



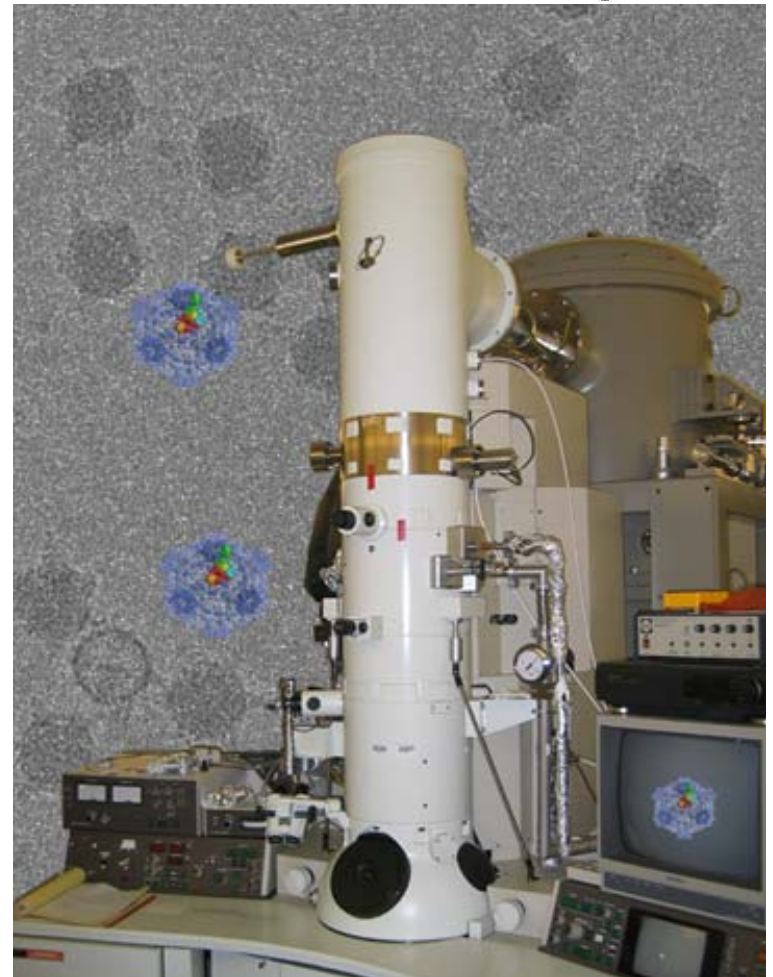
Wah Chiu

Baylor College of Medicine

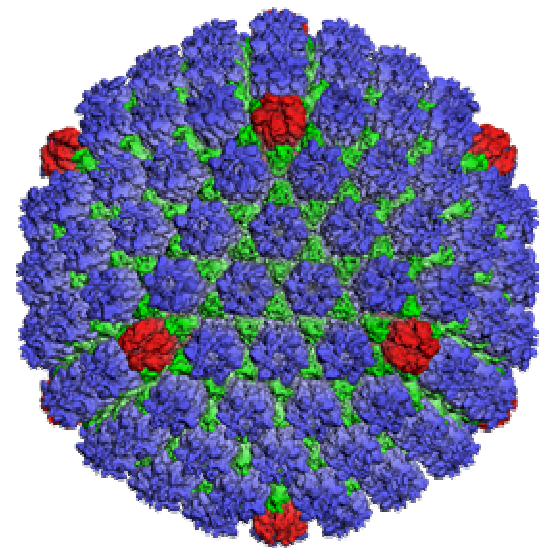
wah@bcm.tmc.edu



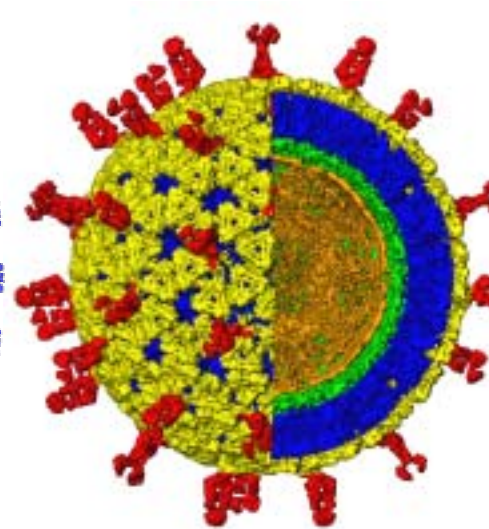
**National Center for
Research Resources**



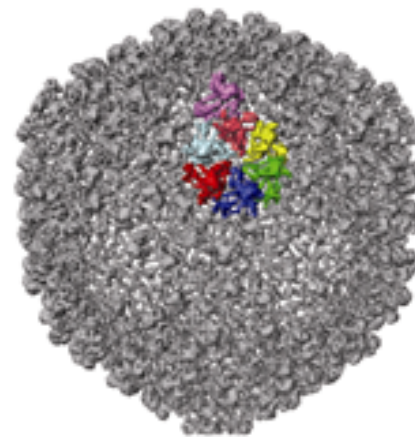
Subnanometer Structures Solved at NCMI by Cryo-EM



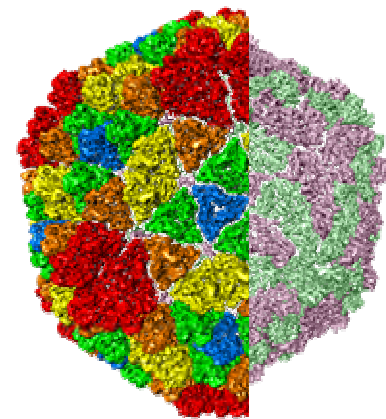
HSV, 195MDa



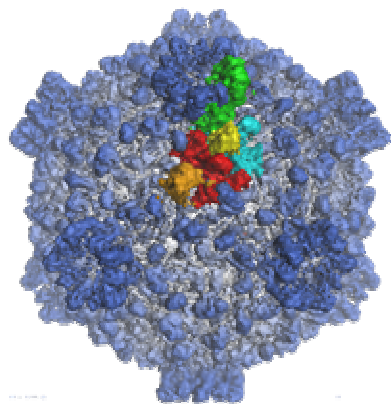
Rotavirus, 100MDa



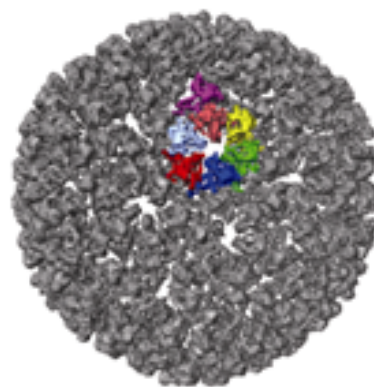
P22 phage, 40 MDa



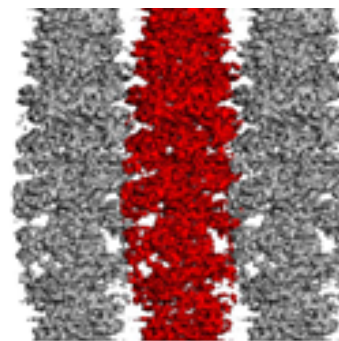
RDV, 75 MDa



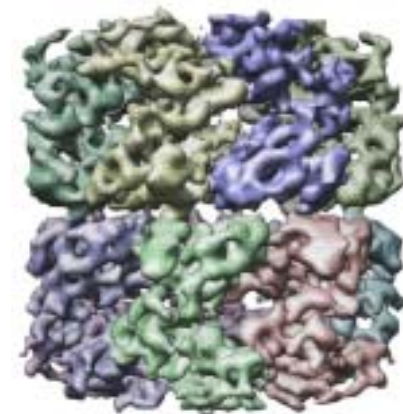
CPV, 55MDa



P22 procapsid
20 MDa

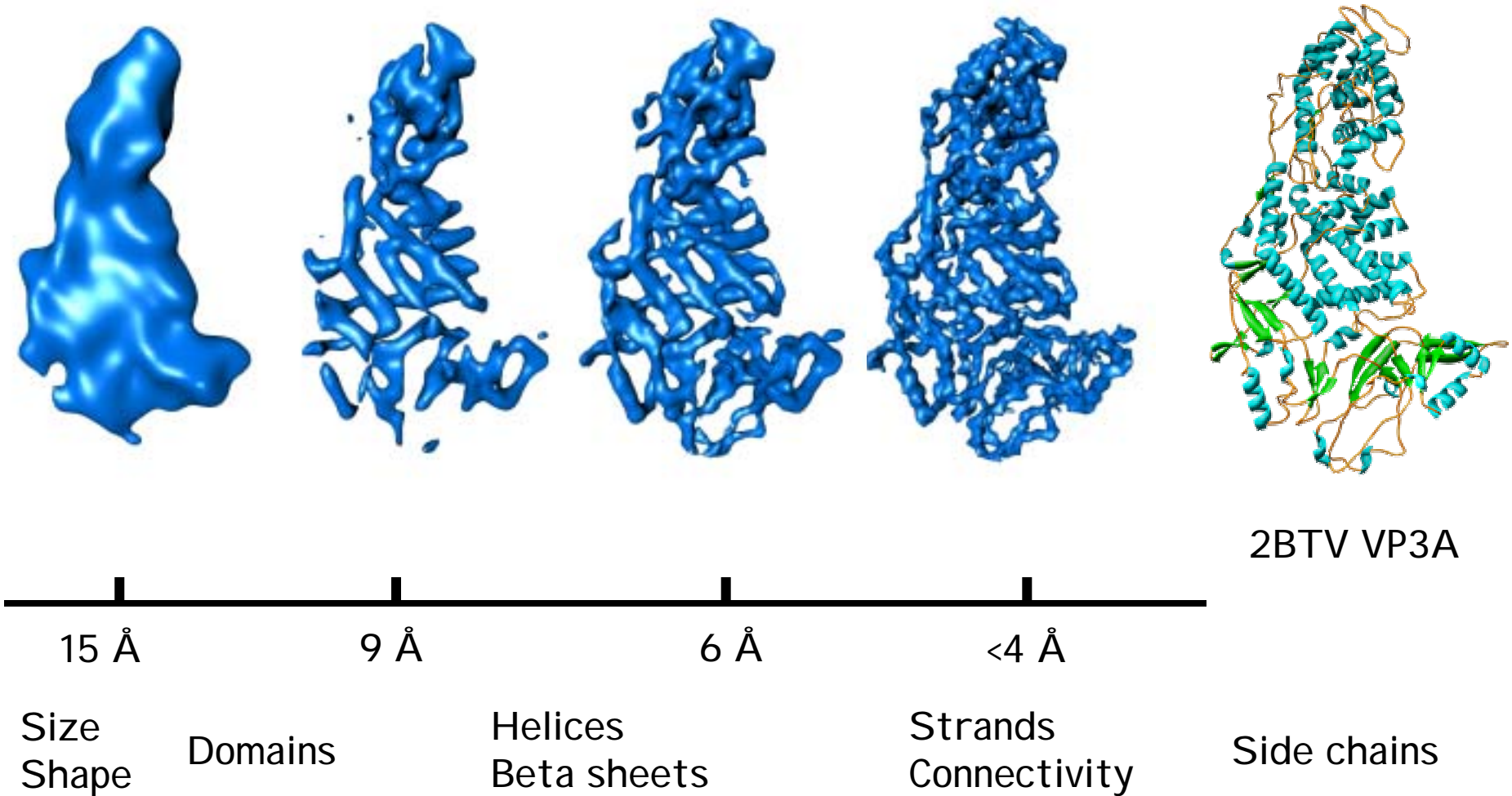


Actin Bundle
2.2 MDa/A.U.



GroEL (4x mag)
800 KDa

Structure at Different Resolutions



Cryo-EM Structures at Sub-nanometer Resolutions

- How reliable is the structure ?
- What information can be derived ?
- How far can the resolution be extended ?

GroEL : Protein Folding Machine

Steven Ludtke

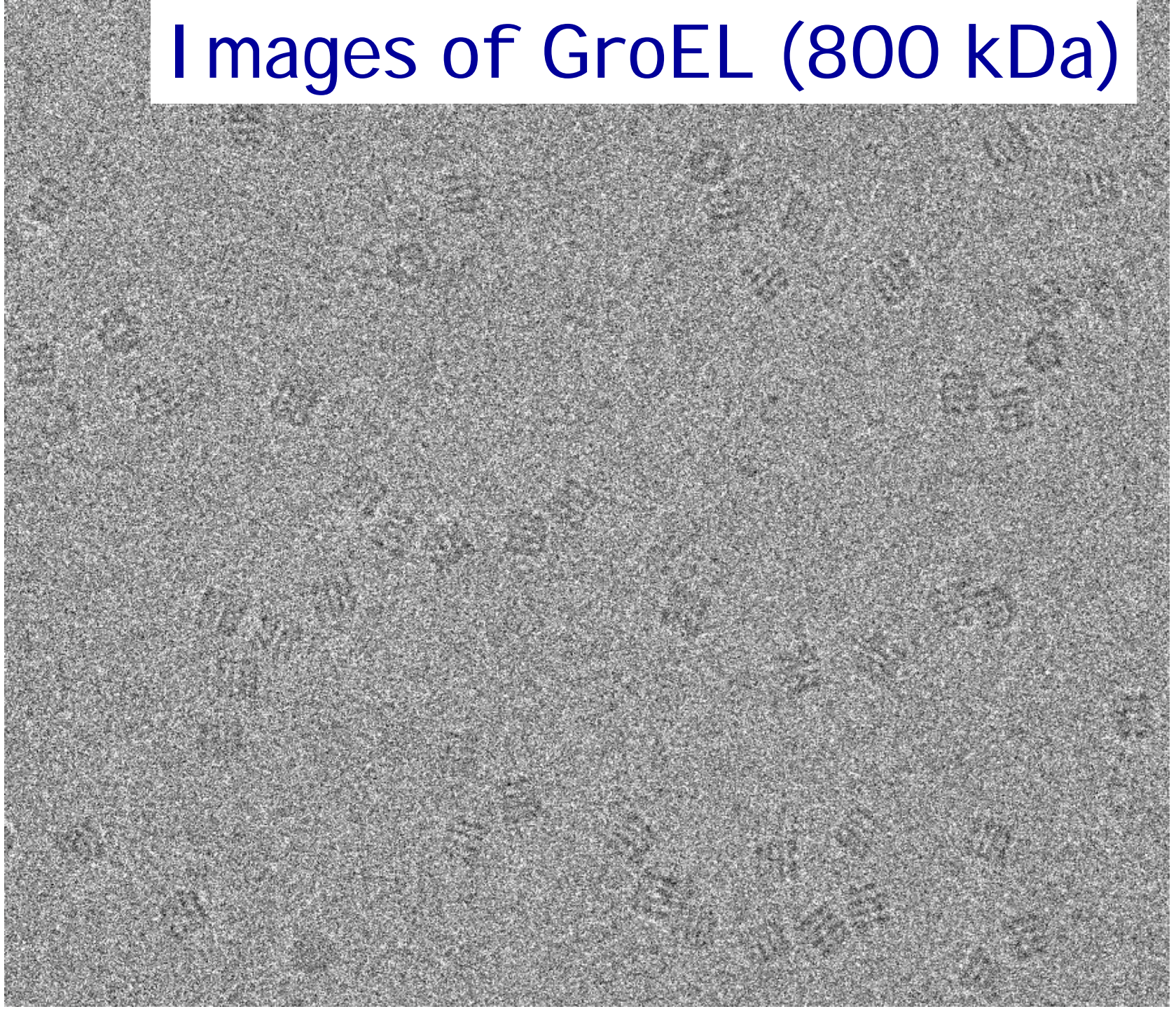
Dong-Hua Chen

Jiu-li Song (UTSW)

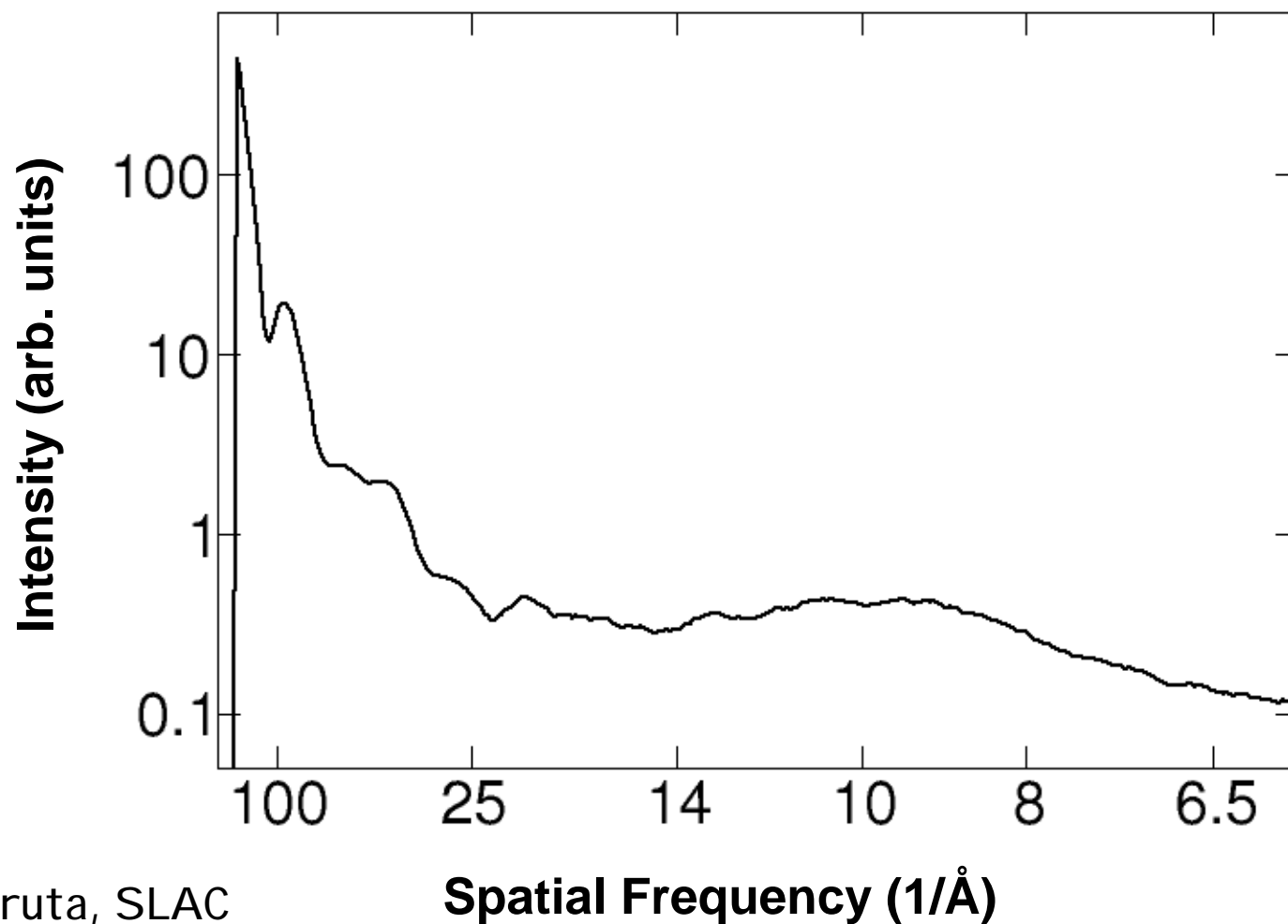
David Chuang (UTSW)

Ludtke, S. J., Chen, D. H., Song, J. L., Chuang, D. T., and Chiu, W. (2004).
Seeing GroEL at 6 Å resolution by single particle electron cryomicroscopy.
Structure (Camb) 12, 1129-1136.

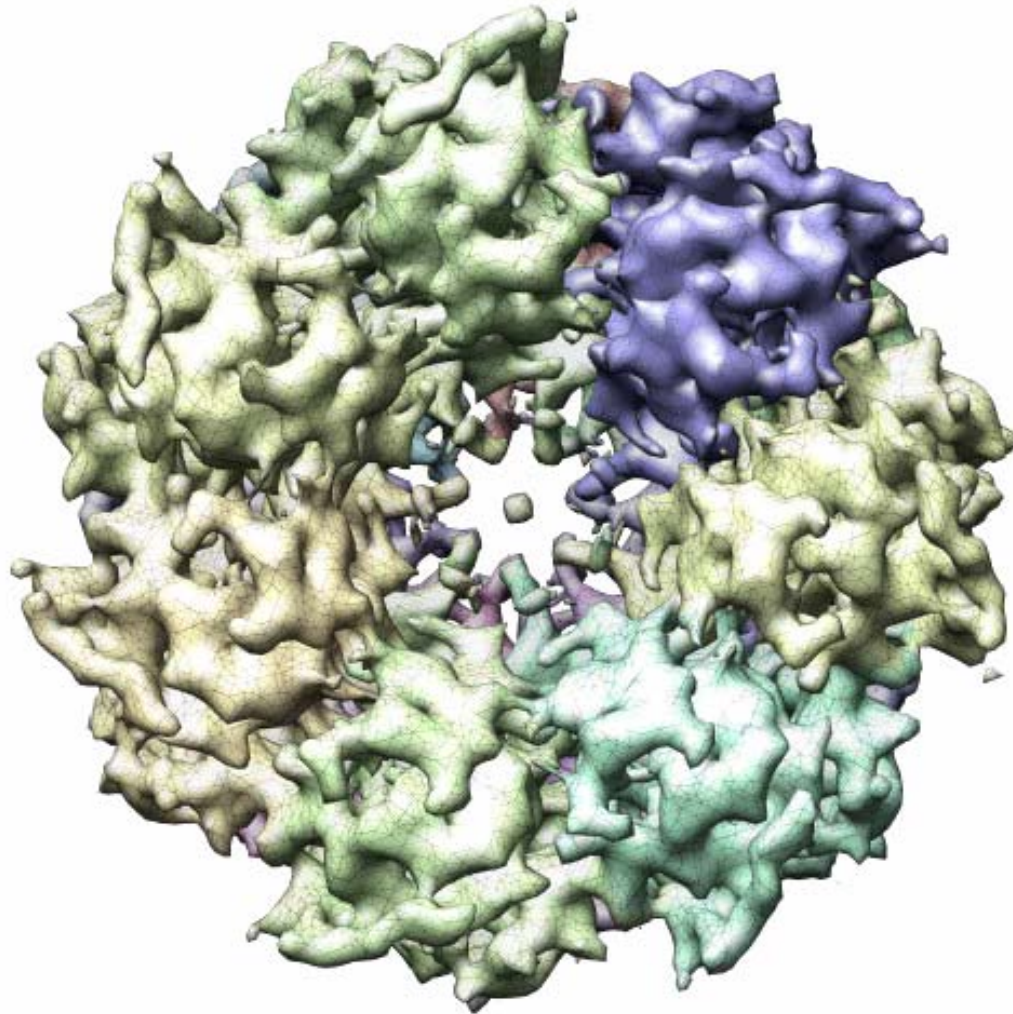
Images of GroEL (800 kDa)



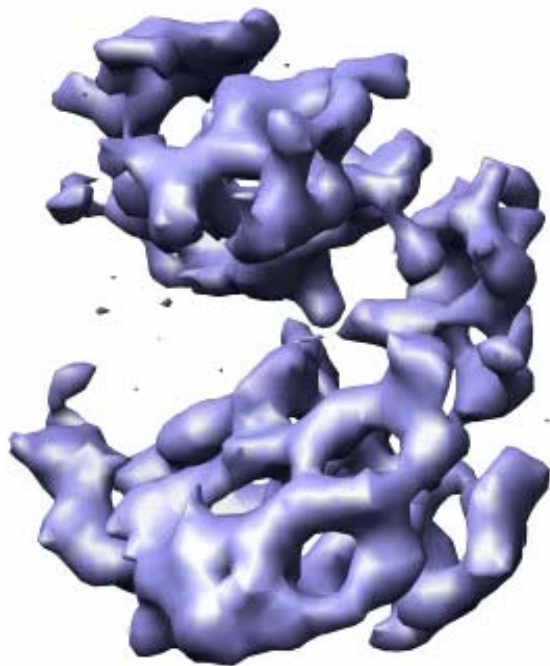
X-Ray Solution Scattering



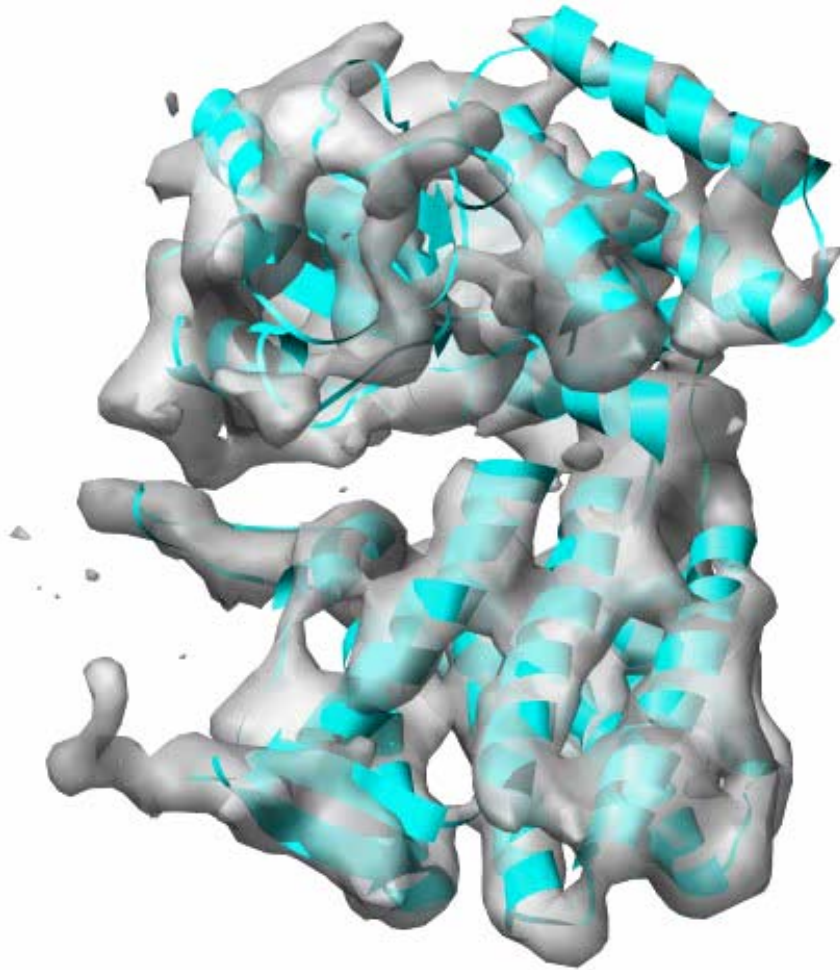
GroEL Cryo-EM Map at 6 Å



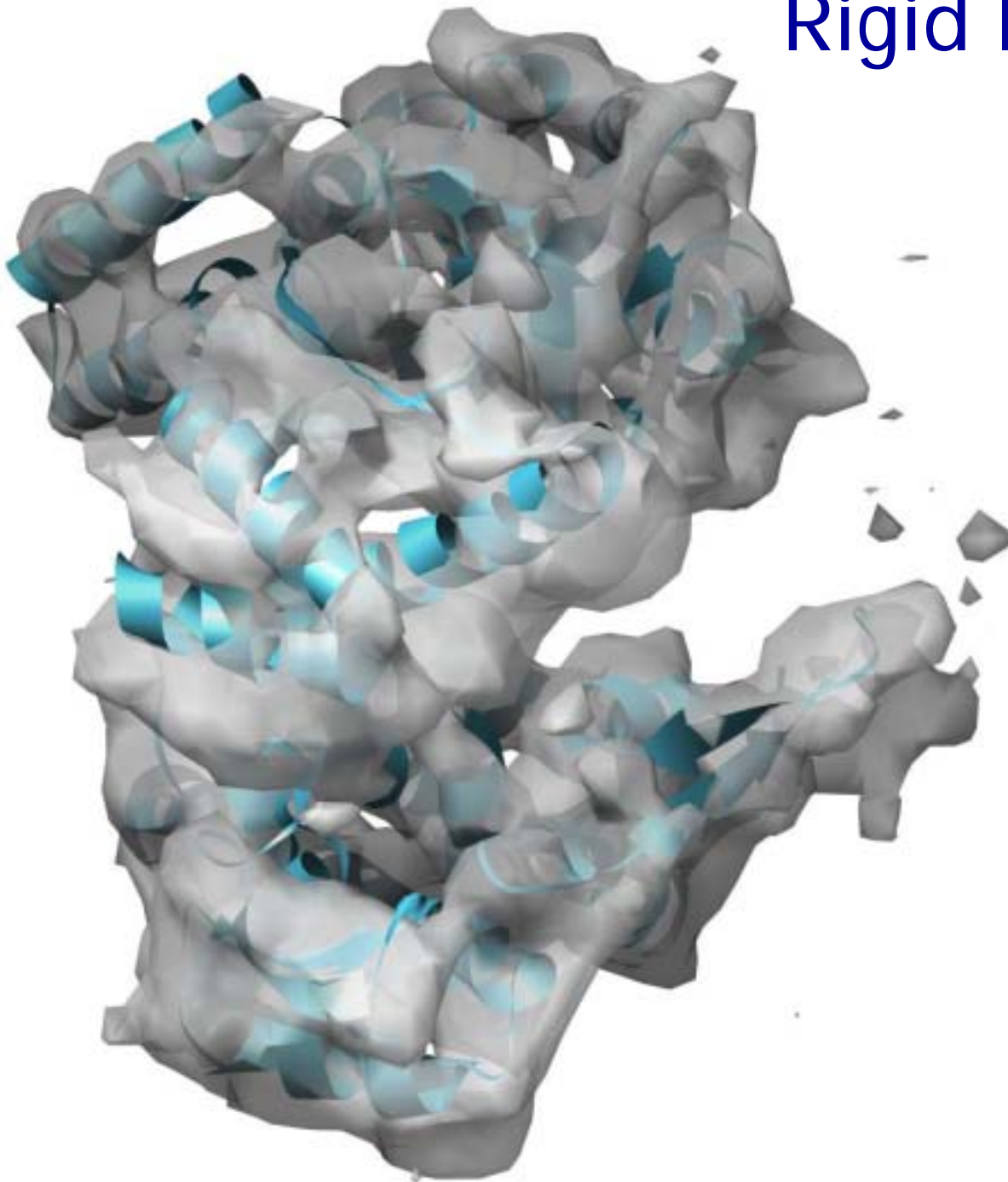
Matching Cryo-EM and Crystal Structures



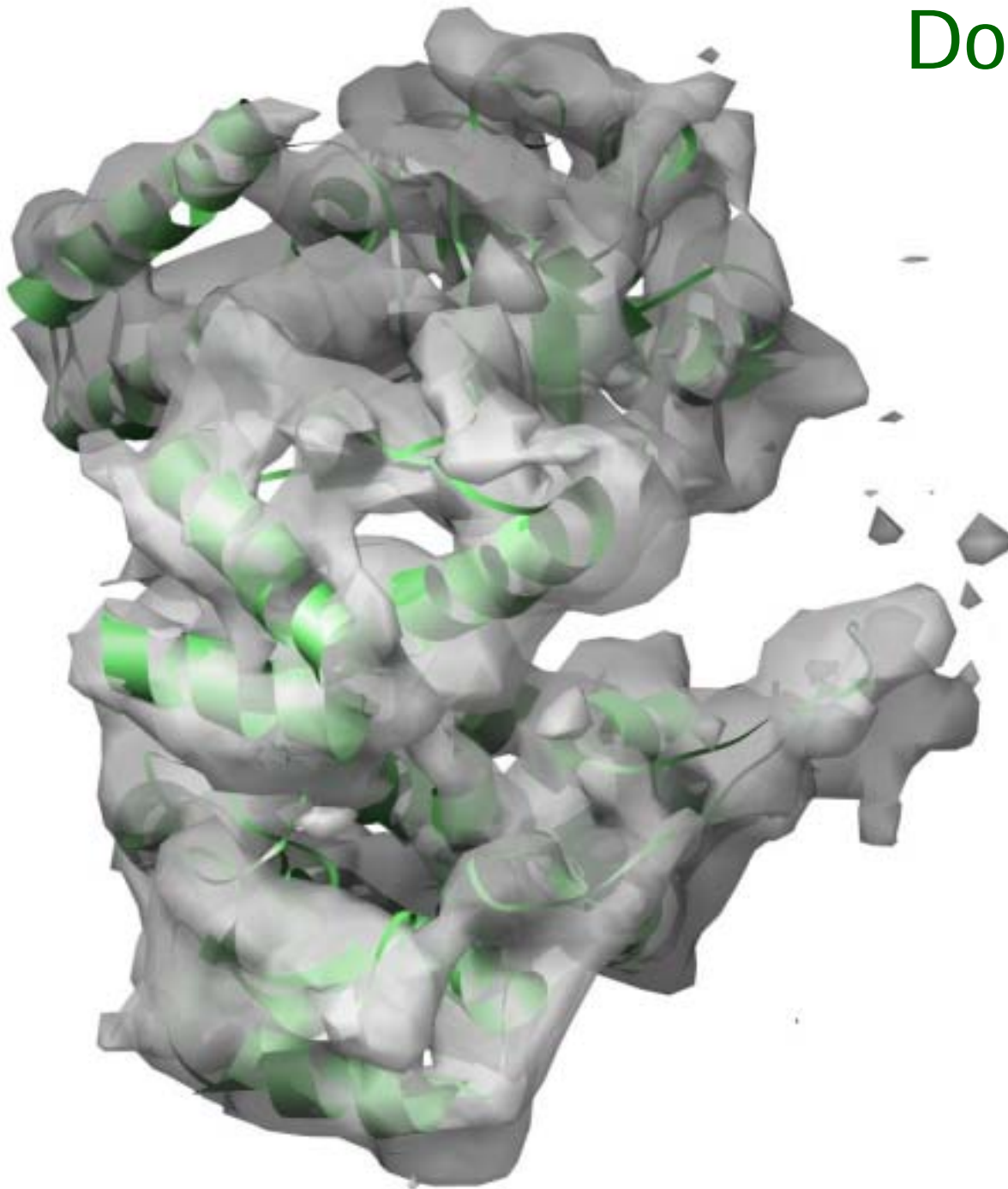
Matching Cryo-EM and Crystal Structures



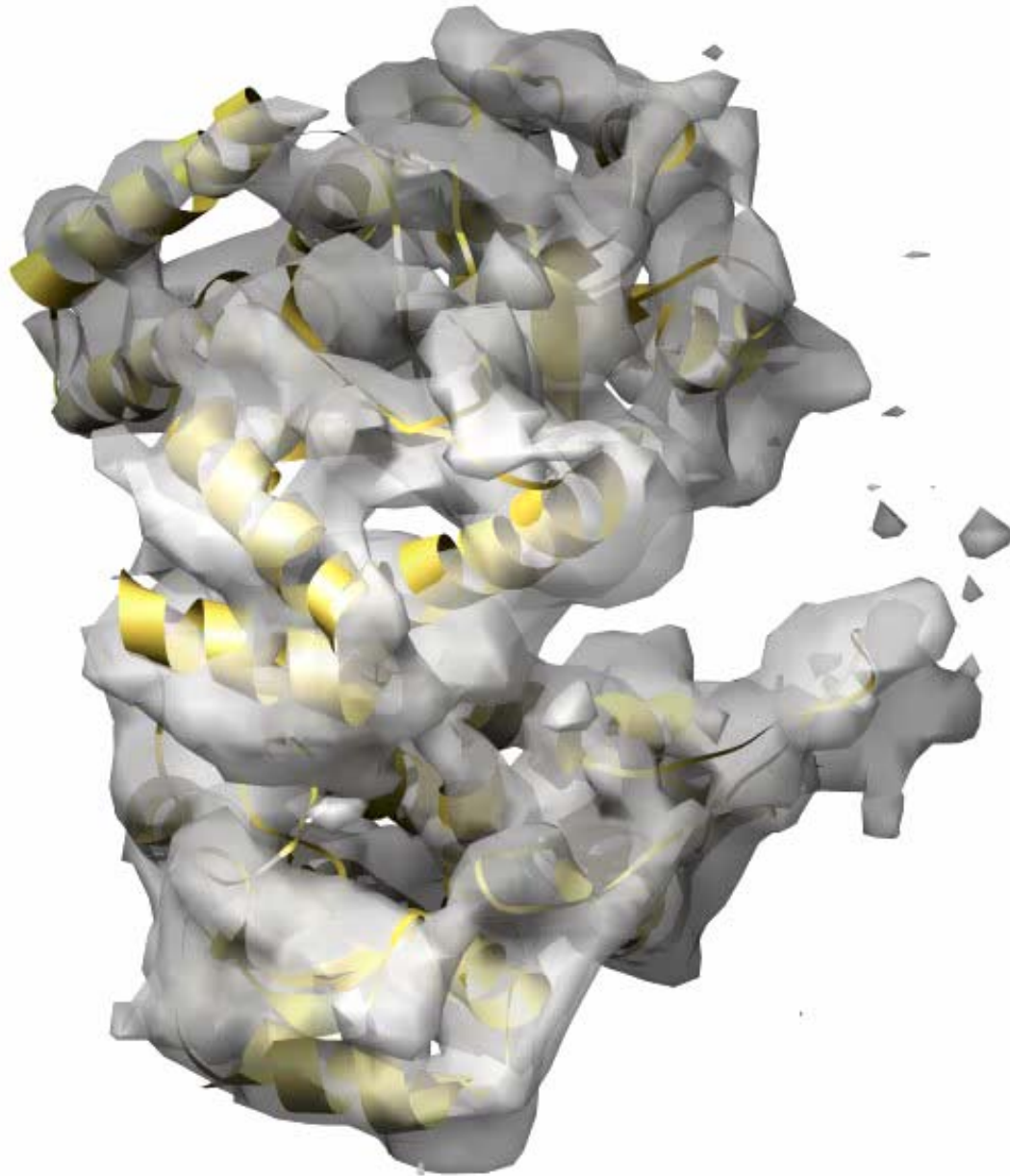
Rigid Body Fit



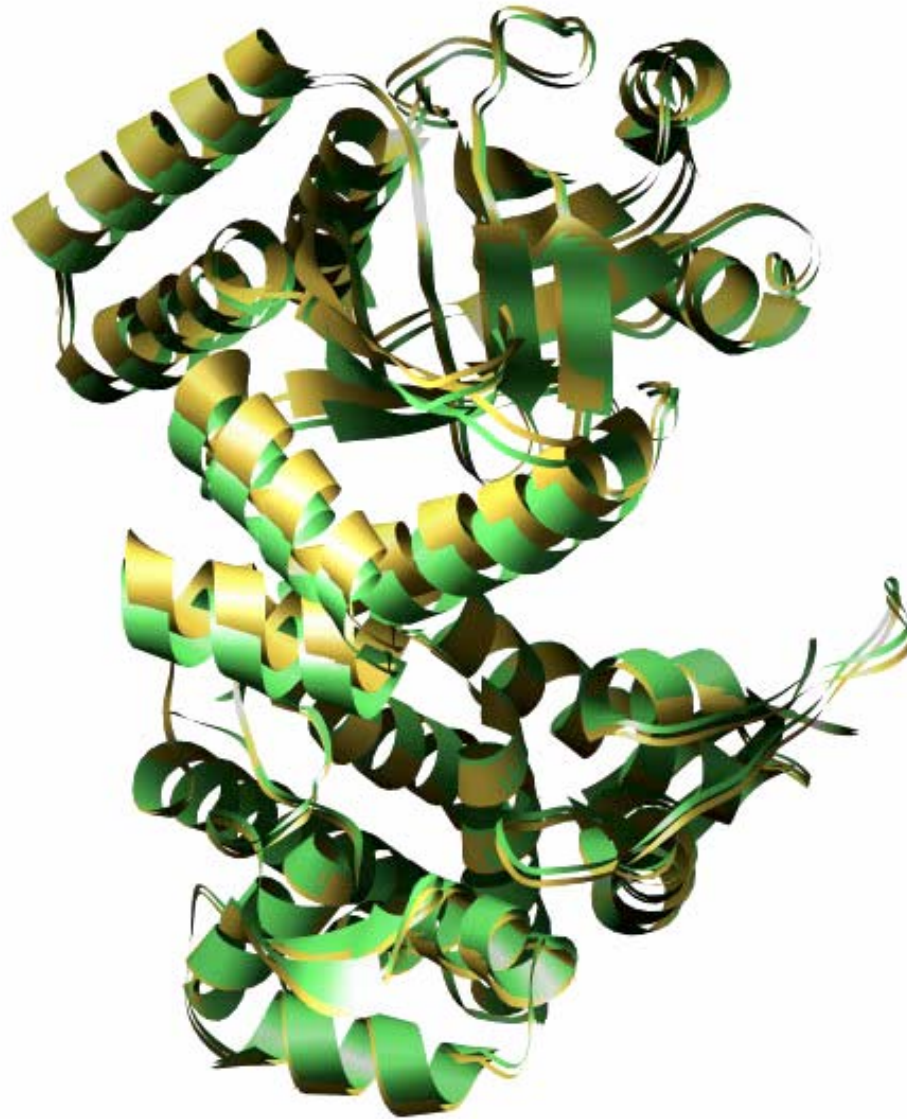
Domain Fits



7 GroEL Crystal Structures



Match between Cryo-EM and Crystal Models



Conclusions - GroEL

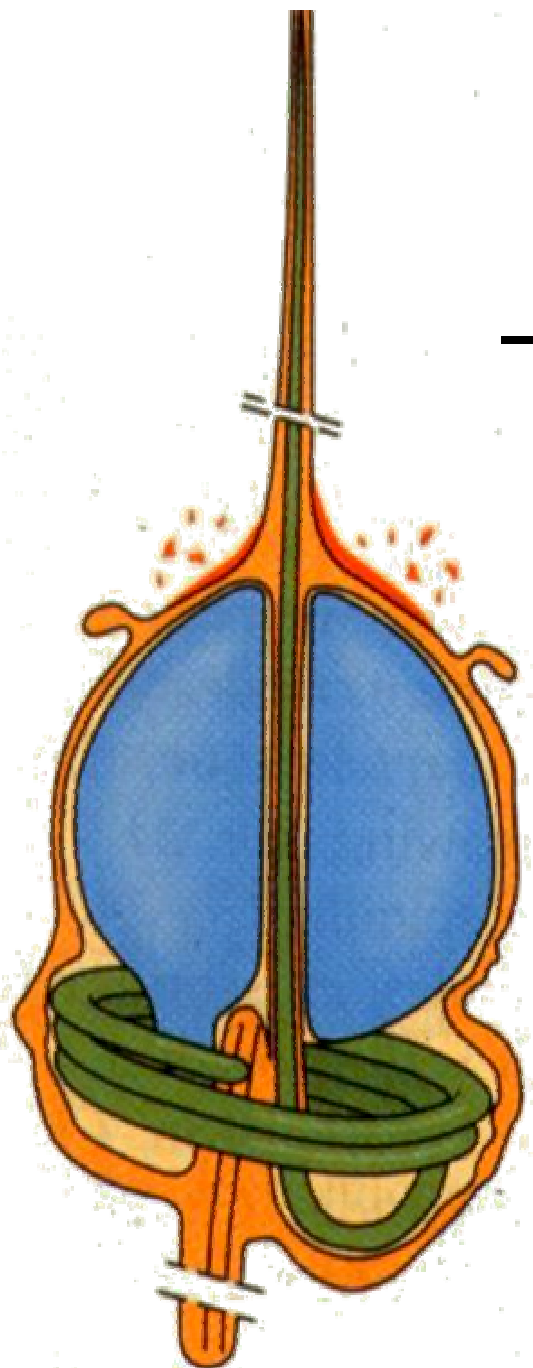
- Equatorial domain matches well, confirms veracity of technique
- Intermediate domain shows ~ 3 Å shifts compared to crystal structure
- Apical domain is consistent with one crystal subunit structure, evidence for dynamics
- C terminus 'missing' density in crystal structure observed in cryoEM map

Acrosomal Bundle: Biological Spring

Michael Schmid
Misha Sherman
Joanita Jakana
Matthew Dougherty

Paul Matsudaira (Whitehead, MIT)

Schmid et al Nature 2004 in press



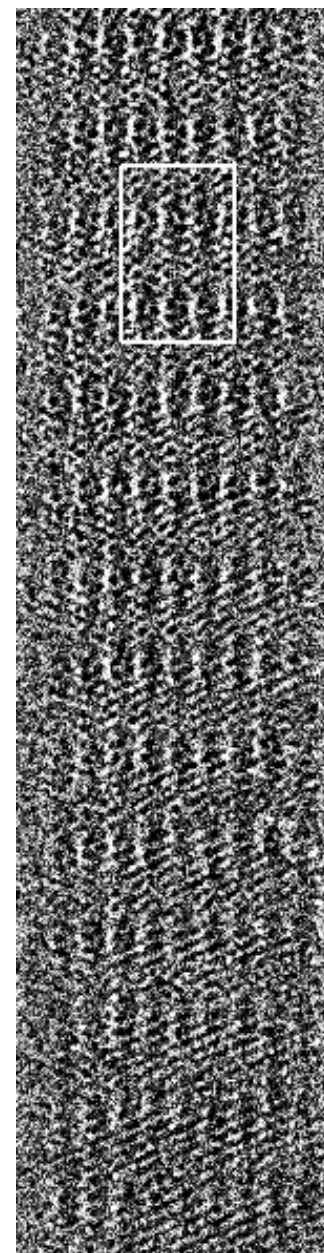
scruin
102kDa

actin
42kDa

calmodulin
24kDa

1:1:1

Cryo-EM image



Schematic of Acrosomal Bundle

"hexagonally" packed

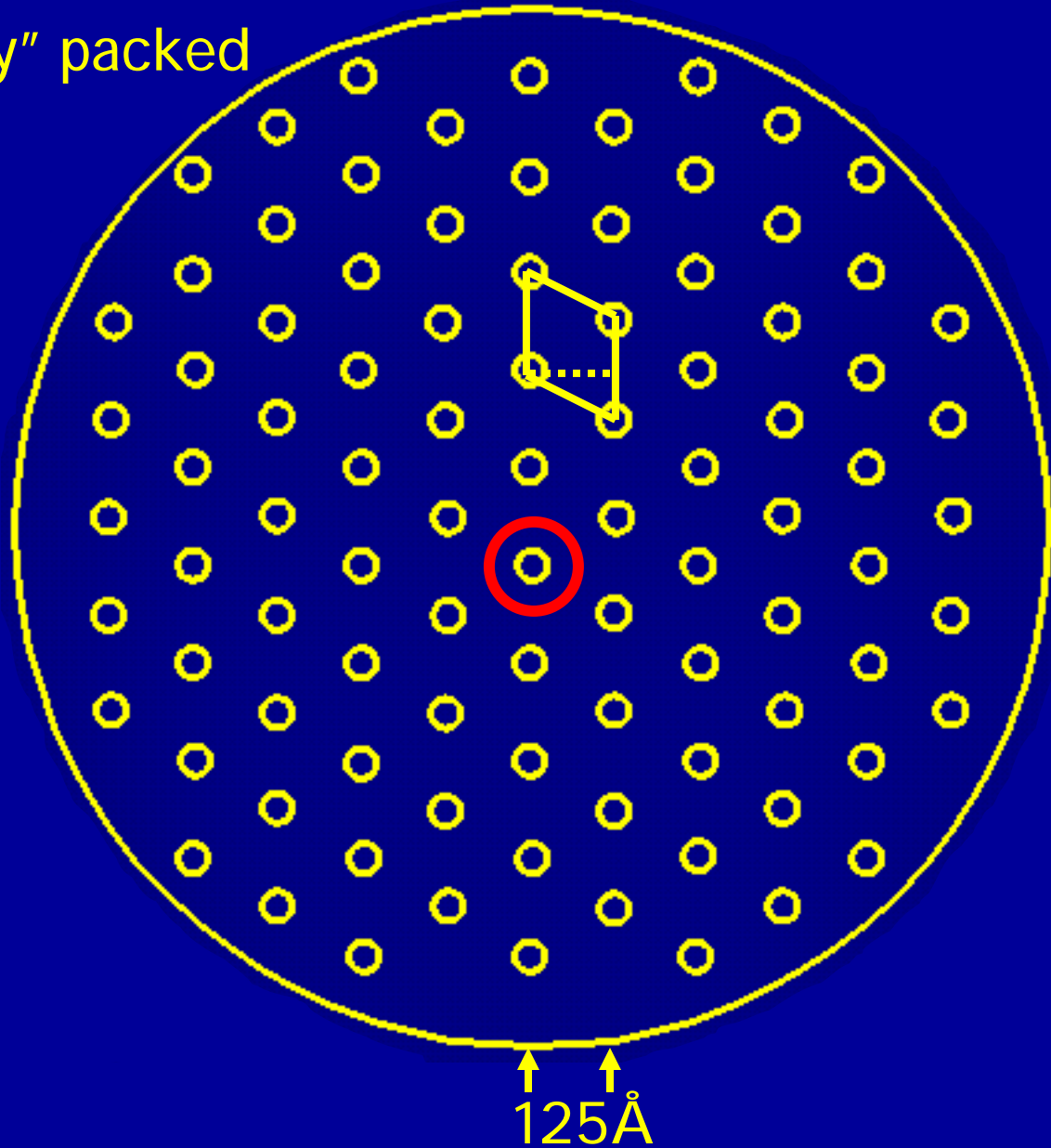
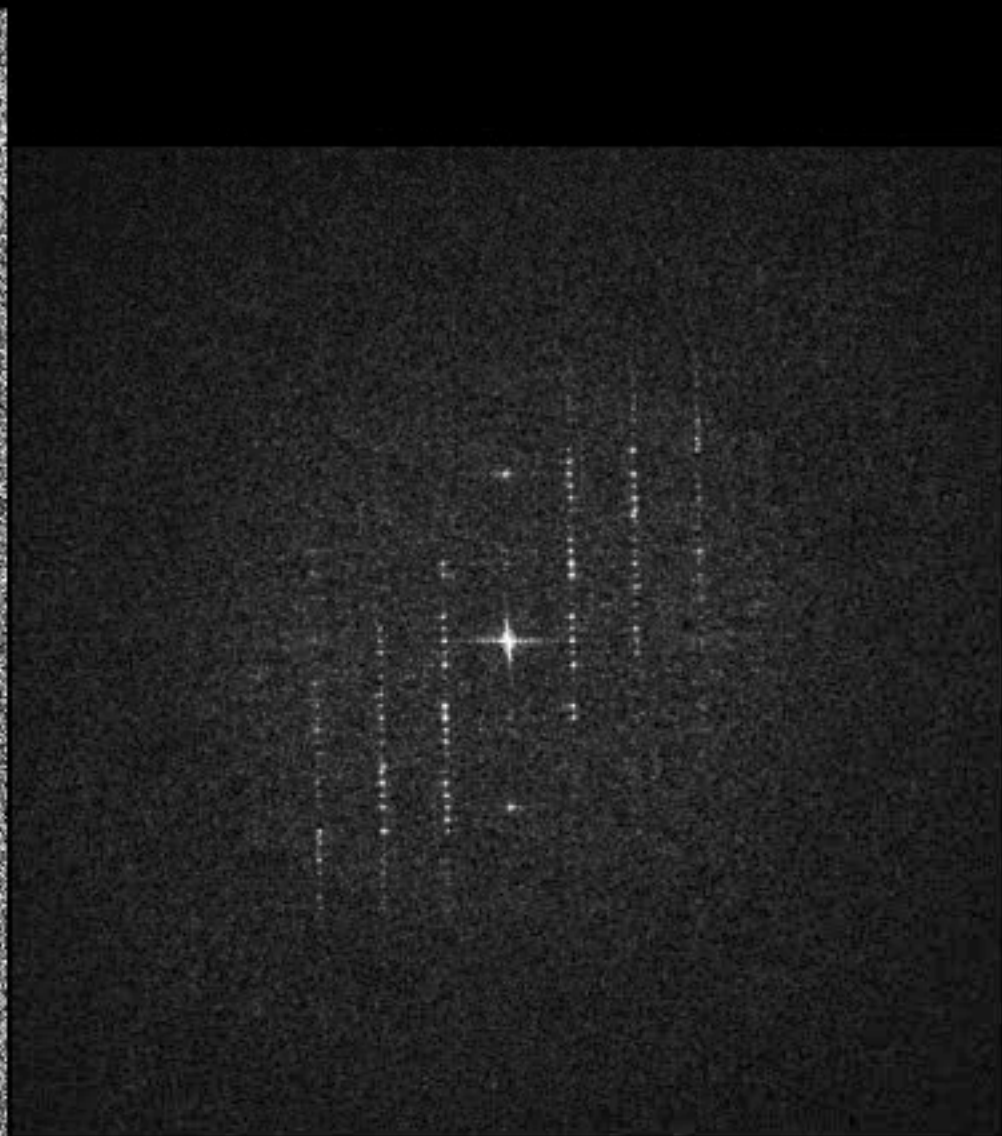
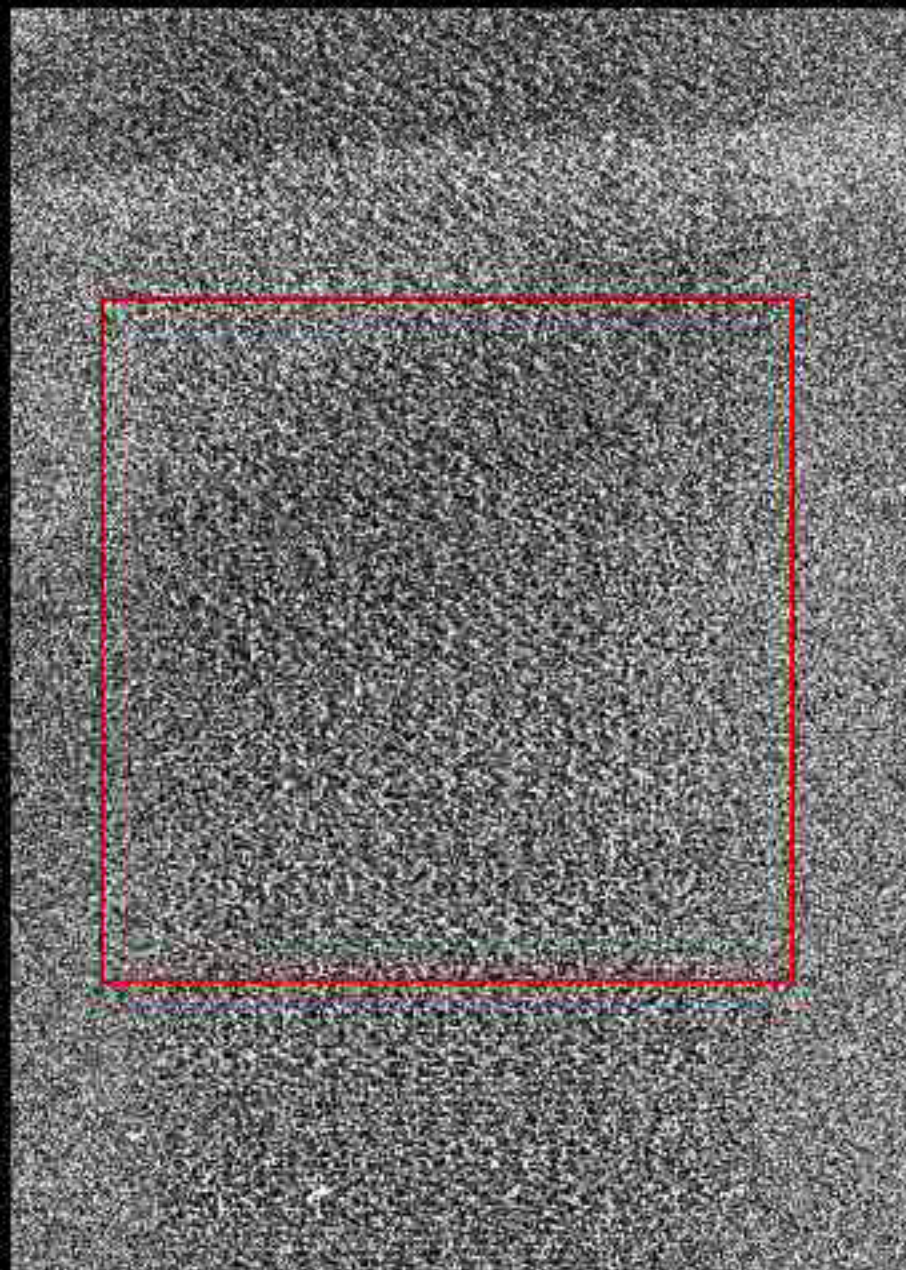


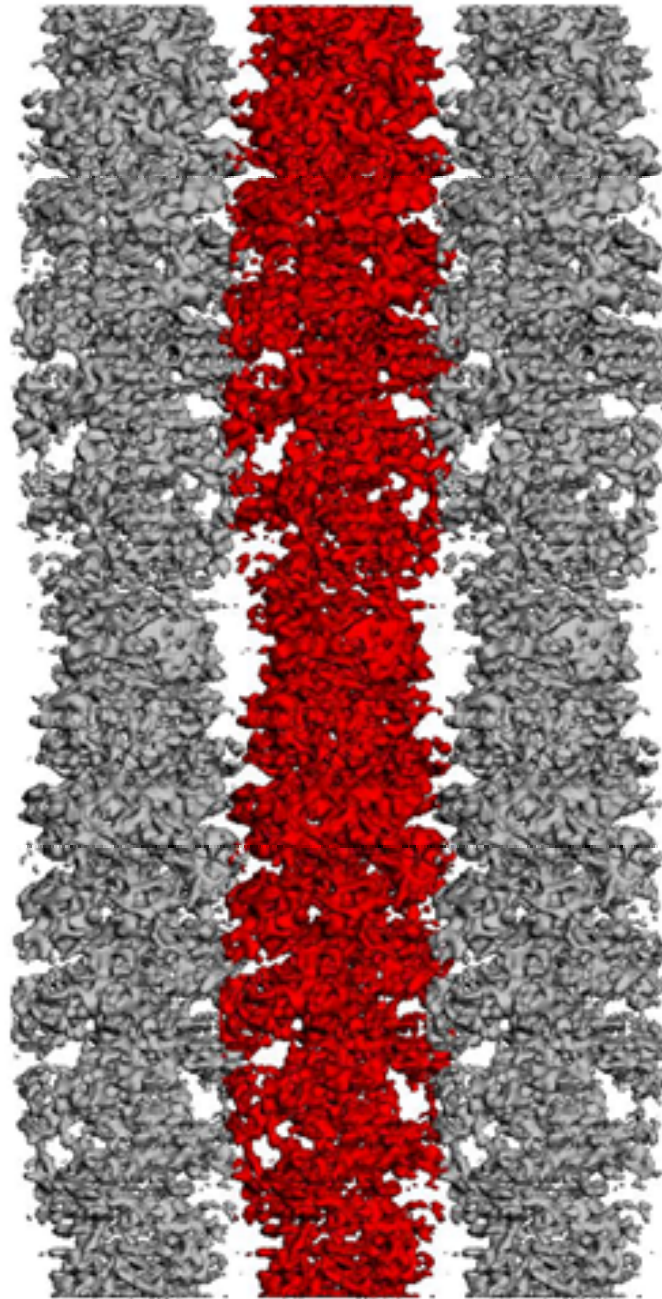
Image and Computed Diffraction Along a Bundle



9.5 Å Map of the Acrosomal Bundle

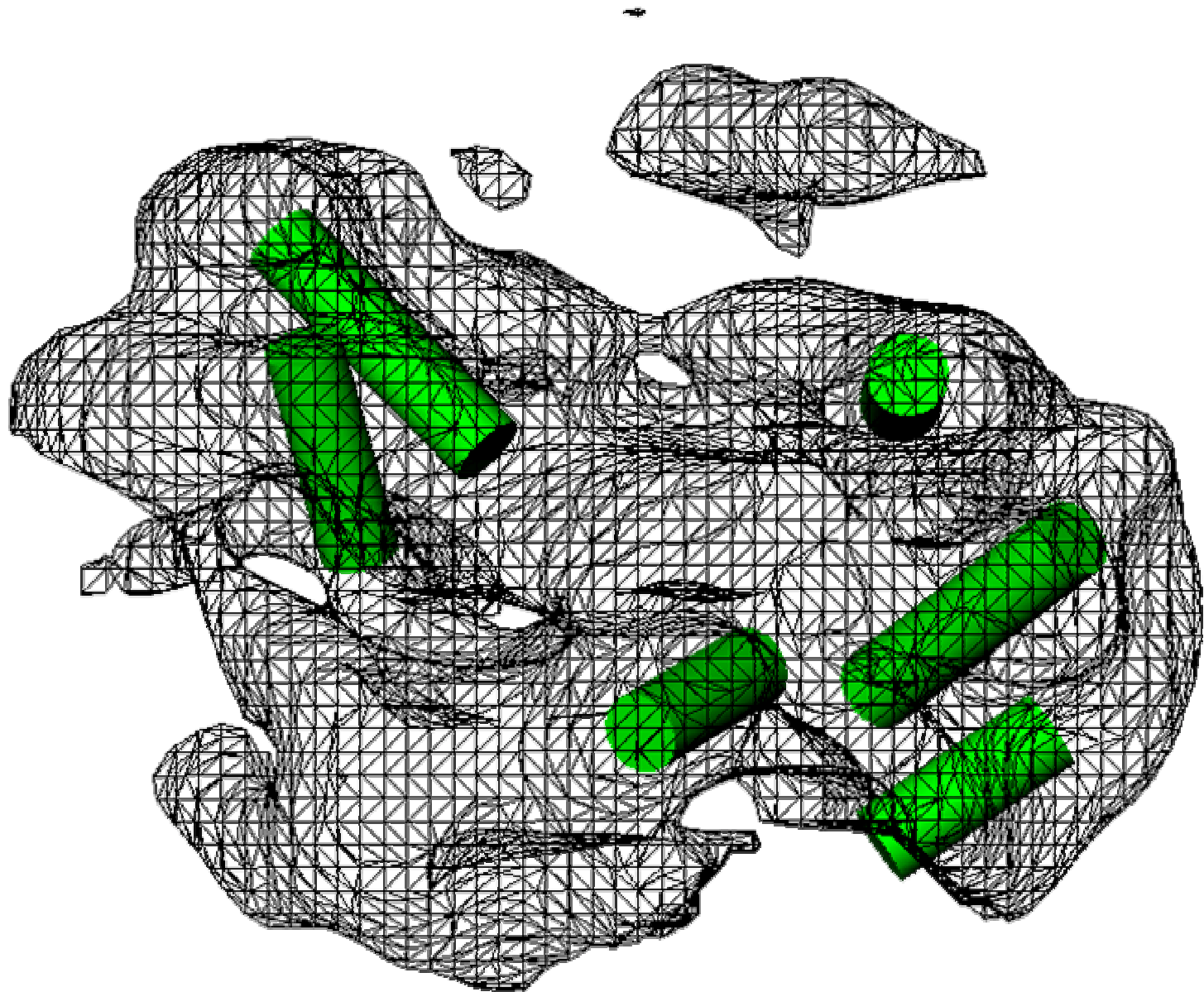
acrosomal bundle

3 filaments



Space Group $P2_1$
146x146x765Å
28 actin-scrutin
13 helical turns

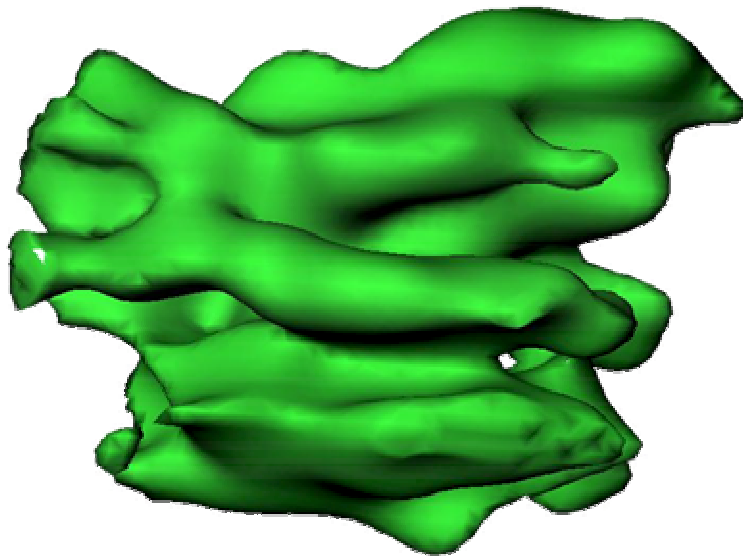
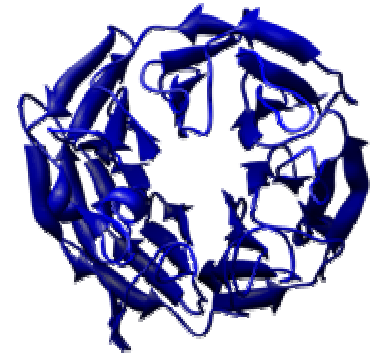
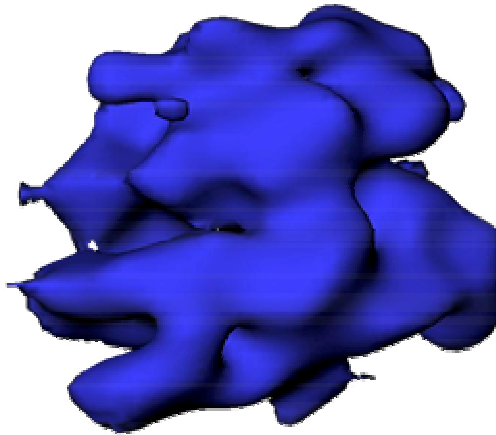
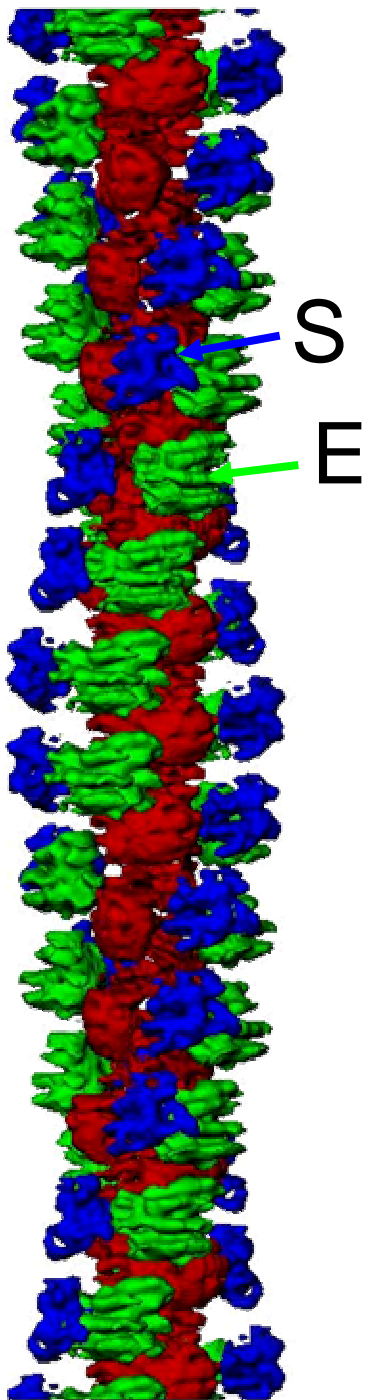
Refined Actin Structure in the Bundle



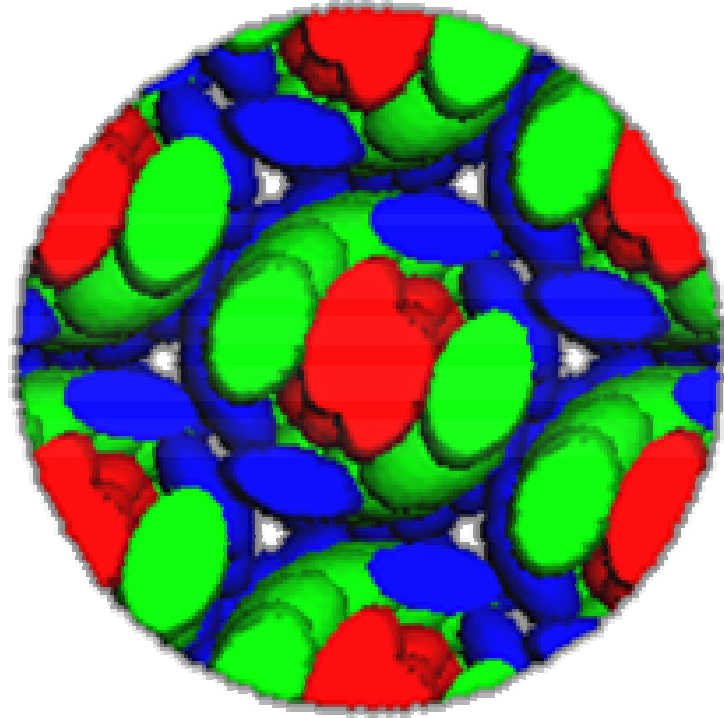
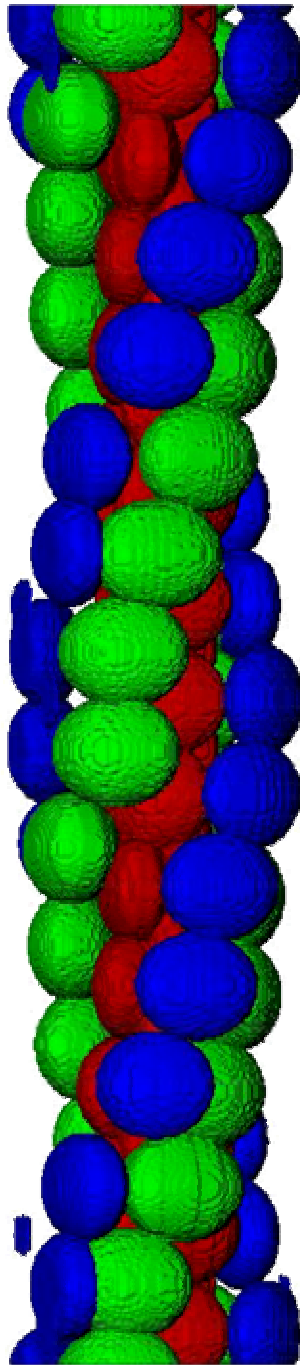
transformation from
perfect F-actin helix
to acrosomal actin



Scruin Domains



Interactions



Summary

- Highest resolution structure of actin molecule seen in a native biological environment
- Hydrophobic plug of actin is clearly seen
- Actin is not organized as a true helical filament in the bundle
- Switch from coiled to extended form involves small changes in actin orientation in the filament
- Scruin-actin interactions are delineated

The Future in Cryo-EM

- Higher 'routine' resolution (3-4 Å)
- Polypeptide backbone trace in single particle reconstructions
- Techniques to address dynamics of individual subunits
- Higher throughputs in sub-nanometer resolution structure determination